Sitosterolemia: platelets on high-sterol diet

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In this issue of Blood, Kanaji et al have identified the cellular mechanisms responsible for the bleeding abnormality and macrothrombocytopenia associated with sitosterolemia. Sitosterolemia is a rare inherited lipid metabolic disorder characterized by the presence of xanthomas, premature coronary artery disease, and atherosclerotic disease. The hallmark of sitosterolemia is diagnostically elevated plasma levels of dietary plant sterols (eg, sitosterol), which is found in high concentrations in olives, avocados, and pecan nuts. Sitosterolemia is caused by mutations in 2 genes, ABCG5 and ABCG8, tandemly located in a head-to-head orientation on chromosome 2p21. ABCG5 and ABCG8 encode adenosine triphosphate–binding cassette transporters, ABCG5 and ABCG8 (also called sterolin-1 and -2, respectively), which are expressed at the highest levels in hepatocytes and enterocytes in humans and mice. Deficiency in ABCG5 or ABCG8 cause increased intestinal absorption and decreased biliary excretion, and levels of Abcg5 and Abcg8 mRNAs increase in mice within 1 week of beginning a high-cholesterol diet. Thus, ABCG5 and ABCG8 form a functional heterodimer that needs to be expressed coordinately and mediates efflux of dietary sterol from the small intestine, thus protecting humans from sterol accumulation.

Mediterranean stomatocytosis/macrothrombocytopenia has been identified as the hematological presentation of sitosterolemia. The disorder is characterized by stomatocytosis, hemolysis, large platelets, splenomegaly, and bleeding. Consistently, mice genetically modified to lack Abcg5 and “thrombocytopenia and cardiomyopathy” (trac) mice presenting a natural nonsense mutation W462X in Abcg5, have severe macrothrombocytopenia and strong bleeding tendency. Both Abcg5−/− and Abcg8trac/trac mice have splenomegaly and increased counts of megakaryocyte progenitors in their bone marrow and spleen. Megakaryocyte development is defective in both mouse models, with perturbation of the normally highly invaginated demarcation membrane system that is crucial for platelet formation. Hematological parameters are normalized when bone marrow cells from Abcg5−/− mice are transplanted into irradiated wild-type mice or when Abcg5−/− mice are treated with the clinically used intestinal sterol absorption inhibitor ezetimibe. Serum from Abcg5trac/trac mice also inhibits proplatelet formation by wild-type fetal liver–derived megakaryocytes.

Thus, the macrothrombocytopenia associated with Abcg5 deficiency is caused by increased plasma plant sterol levels resulting from defective ABCG5/ABCG8 heterodimer function in the liver and small intestine, and not to intrinsic megakaryocyte defects. The mouse models differ in the severity of the hemolytic anemia because red blood cell counts and their resistance to hemolytic stress are only mildly affected in Abcg5−/− mice, whereas Abcg5trac/trac mice have a mild hemolytic anemia with increased reticulocyte numbers. The differences are likely due to strain background and/or diet effects.

Kanaji et al address the concerns of the previous studies and further decipher the cellular mechanisms responsible for the bleeding abnormality and macrothrombocytopenia in 2 mouse models of sitosterolemia. Abcg5−/− and Abcg8−/− mice were backcrossed to
C57BL/6 background and fed with custom diets containing low or high plant sterols. *Abcg5^{−/−}* and *Abcg8^{−/−}* mice present hematological parameters comparable with those of wild-type mice when fed a low-sterol diet (<0.01% weight/weight), but develop a profound macrothrombocytopenia and hemolytic anemia, accompanied with prolonged bleeding time, when fed a high-sterol diet (1% weight/weight) for 6 weeks. Thus, the observations confirm that the severity of sitosterolemia and its hematological presentation, Mediterranean stomatocytosis/macrothrombocytopenia, depends on both genetic and dietary components.6

By flow cytometry, using the fluorescent sterol dye filipin and gas chromatography–mass spectrometry, Kanaji et al demonstrate that plant sterols incorporate directly into the platelet membrane of *Abcg5^{−/−}* mice fed a high-sterol diet. Sterol incorporation has disruptive effects on lipid asymmetry in *Abcg8^{−/−}* mice fed a high-sterol diet, promoting the generation of platelet-derived microparticles with exposed phosphatidylserine and increased surface-bound fibrogenin despite markedly reduced surface expression of its receptor, the integrin αIβ6β3. Platelets isolated from *Abcg8^{−/−}* mice fed a high-sterol diet have impaired responsiveness to agonist-induced activation and adhere poorly to von Willebrand factor and type I collagen at high shear rates.

Biochemical analysis shows that platelets isolated from *Abcg8^{−/−}* mice fed a high-sterol diet have increased activation of the calcium-activated protease calpain, resulting in loss of the cytoskeletal and scaffold protein filamin A (FlnA) and in shedding of the von Willebrand factor receptor GPIbα subunit from the platelet surface. Plasma samples from sitosterolemia patients have increased levels of glycolcalcin, the carbohydrate-rich portion of GPIbα that can be cleaved by calpain, corroborating the observations in *Abcg5^{−/−}* and *Abcg8^{−/−}* mice. Previous studies have shown that deficiency in the GPIbα-FlnA linkage results in macrothrombocytopenia, poor platelet adhesion, and prolonged bleeding time.9,10

*Abcg8^{−/−}* mice fed a high-sterol diet have increased numbers of bone marrow megakaryocytes compared with *Abcg8^{−/−}* mice fed a low-sterol diet, consistent with the previous mouse models.7,8 Isolated bone marrow megakaryocytes differentiate normally, but have slightly decreased expression of calpain, GPIbα, and FlnA, suggesting that megakaryocytes, similar to platelets, are activated by sterol accumulation.

In conclusion, the study by Kanaji et al demonstrates that the bleeding abnormalities and macrothrombocytopenia associated with sitosterolemia are due to direct plant sterol incorporation into the platelet membrane, resulting in platelet hyperactivation, reduced αIβ6β3 surface expression, loss of the GPIbα-FlnA linkage, microparticle formation, and ultimately poor hemostatic functions (see figure). Many questions remain unanswered. For example, how does sterol accumulation activate calpain? What are the cellular mechanisms responsible for stomatocytic hemolysis? Sitosterolemia is significantly underdiagnosed because it is influenced by both genetic and dietary components, which is particularly relevant in Western societies that consume a high-sterol diet. Whether the observations by Kanaji et al are relevant to coronary artery disease and atherosclerotic disease remains to be elucidated.

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REFERENCES


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Comment on Belcher et al, page 2757

HO-1 and CO: fighters vs sickle cell disease?

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In this issue of Blood, Belcher et al report on the use of pegylated hemoglobin saturated with carbon monoxide (CO) to inhibit vaso-occlusive events in mouse models of sickle cell disease (SCD).1

It has been more than 60 years since sickle cell anemia (SCA) was first characterized at the molecular level by Linus Pauling. SCA occurs when thymine is substituted for adenine in the 6th codon of the beta-globin gene, resulting in a substitution of the hydrophilic aminoacid glutamic acid by the hydrophobic amino acid valine (Hb S). SCD includes SCA and the compound heterozygous sickle hemoglobinopathies. It is one of the most common monogenic diseases worldwide, responsible for over 80% of the significant hemoglobinopathies in the world. Remarkable progress has been achieved in the care of individuals with SCD, including the use of hydroxycarbamide (hydroxyurea), yet the only definite therapy remains limited to stem cell transplantation,
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